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14. ABSTRACT This project is based on the premise of developing a new method to identify and establish the presence of phenotypic diversity/genetic heterogeneity in Gulf War Illness patients. In our previous research, we successfully established a link between genomic instability (displayed as elevated frequencies of non clonal chromosome aberrations or NCCAs) and cancer progression. In this DOD supported grant during the current study period, we have analyzed GWI patient samples using multiple-color spectral karyotype profiling or SKY and expression profiles. Elevated NCCAs have been observed in all GWI patient samples tested so far representing a highly statistically significant finding. We have tested 15 GWI patients and 35 controls to date. We believe that our methodology represents a means to identify a clinical biomarker based on phenotypic diversity/heterogeneity in GWI patients. We are developing a theory that links stress to the increased genome instability observed. This new concept and findings will support future translational projects that will result in vastly improving diagnosis and assessment of GWI patients.					
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Introduction

The objective of this proposal is to determine whether there is a link between genome instability (reflected as elevated frequencies of NCCAs) and GWI. Patient samples (blood) are being directly analyzed using molecular cytogenetic analyses and gene expression studies. *The central hypothesis of this proposal is that patients with GWI will display high levels of genome instability, particularly when challenged by various toxic agents, which can be stochastically linked to various molecular pathways and is the potential basis for diverse clinical symptoms that are seen in GWI.*

Specific Aim 1: Establish a method to determine levels of genome variation/NCCAs in blood cells (lymphocytes) to monitor overall genetic instability in patients with GWI.

Specific Aim 2: Link high levels of genome instability to various gene level alterations or molecular pathways illustrated by gene expression studies and copy number variation analysis.

Purpose and scope of the research effort: These specific aims will systematically link genomic instability as detected in the blood cells of veterans with detectable genetic alterations. It is anticipated that this study will form the basis of new methodology that can be translated to prescreen veterans for the likelihood of developing GWI or used to diagnose GWI in veteran populations.

Body

I. Continue building up the strong linkage between GWI patients and elevated genome instability

An additional 5 cases of GWI have been analyzed. The frequencies of NCCA range from 17% to 33%, with an average of 26.5%. These data are slightly higher than the previously determined average of 24.2% based on 10 cases. These data have further demonstrated the linkage of GWI and genome instability. Additional cytogenetic analyses have shown that, despite the elevated frequencies of NCCAs, there is no specific pattern observed in terms of which specific types of chromosomal aberrations dominate.

To date, our lab has tested 15 GWI patients and 35 controls (both from military and non-military populations). Current data strongly support our hypothesis that GWI patients display significantly elevated genome instability. We are now in the process of writing the manuscript for initial publication.

II. Re-classify military control group

Last year, we noticed that the NCCA frequencies within the military population are slightly higher than non-military population (3.16% vs. 1.5%). To understand this difference, we compared the military control patient profiles. It turns out; some cases within the control group do have some medical conditions. For example, control #23 is a cancer survivor who displays much higher frequencies of NCCAs. Interestingly, for a few cases that also display higher NCCAs, these individuals display chronic fatigue. In a separate study, we compared the overall level of NCCAs for non-military patients (n=15) with chronic fatigue syndrome, and observed increased frequencies from them as well. Together, we realized that we needed to obtain better control cases from VA. The similar links (with lower frequencies compared with GWI) between CFS and genome instability is highly significant, as it also can offer the understanding behind the overlap of these two conditions. As a result we need to continue collecting additional samples that represent true normal military controls without concomitant disease conditions.

III. Search for the common link between elevated genome instability and diverse gene expression patterns is ready for a final analysis

To finish the project, we will perform global gene expression studies on the samples collected thus far. One of the biggest challenges is to understand the meaning behind observed diverse gene expression patterns. A broad spectrum of gene expression changes has been associated with nearly each GWI gene expression study that searches for signature expression patterns. It is therefore necessary to have a common theoretical framework to synthesize the data from the field.

We have identified a broad range of karyotypic change in GWI patients. These karyotypic changes include broadly varying karyotypic abnormalities within the population and non-clonal alterations in individual patients. How then can this data be reconciled? To answer this question dynamic transcriptomic and karyotypic profiles must be compared, however to date no platform to complete this complicated analysis has been developed.

To develop this much needed platform to integrate genomic instability and transcriptomic changes we have used a cell culture model of spontaneous immortalization. This system has well defined changes in karyotype population based patterns even though each run of immortalization results in unique karyotypic changes. Phrased differently this system transitions from a karyotypically stable near normal population to a highly diverse population prior to immortalization and then achieves stability following immortalization. Using this system we recently reported that non-linear karyotypic alteration (seen in GWI) leads to broad changes in transcriptome profiles. The transcriptome change that occurs during the process is highly variable. Interestingly we also found that replicate transcriptome measurements have more variance in samples with increased levels of genomic instability compared to those without. This data suggests that each karyotype results in a unique expression profile. The platform developed in this publication will allow us to link the broad ranging karyotypic changes measured in GWI to the broad spectrum of expression changes linked to GWI. In addition we have linked various types of stress to karyotypic change. The linkage between stress, karyotypic change and transcriptome dynamics likely explains why such a huge number of potential causative agents has been linked to GWI, and reinforces the importance of measuring karyotypic alterations in GWI patients.

Such analysis has been published this year, which can serve as a platform to analyze our expression data for GWI. We have spent significant time in the past year pushing this concept.

Key Research Accomplishments

1. Continued data accumulation supporting the strong linkage between elevated genome instability and GWI.
2. Currently a manuscript is in preparation for publication. This paper will compare Gulf War Illness, Chronic Fatigue Syndrome and control groups.
3. Invited Presentation at the 2013 National Gulf War Illness Advisory Committee (VA) in Washington D.C.

4. Established the linkage between genome alteration and gene expression dynamics. Such platform will be used to understand the gene expression profile.
5. Recently, more of our manuscripts have been published and the DOD's support has been cited. The following papers have been published during the reporting period:
 1. Stevens JB, Liu G, Abdallah BY, Horne SD, Ye KJ, Bremer SW, Ye CJ, Krawetz SA, Heng HH. Unstable genomes elevate transcriptome dynamics. *Int J Cancer*. 2013 Oct 10. doi: 10.1002/ijc.28531. [Epub ahead of print] PubMed PMID: 24122714.
 2. Abdallah BY, Horne SD, Stevens JB, Liu G, Ying AY, Vanderhyden B, Krawetz SA, Gorelick R, Heng HH. Single cell heterogeneity: Why unstable genomes are incompatible with average profiles. *Cell Cycle*. 2013 Oct 1;12(23). [Epub ahead of print] PubMed PMID: 24091732.
 3. Heng HH, Bremer SW, Stevens JB, Horne SD, Liu G, Abdallah BY, Ye KJ, Ye CJ. Chromosomal instability (CIN): what it is and why it is crucial to cancer evolution. *Cancer Metastasis Rev*. 2013 Apr 19. [Epub ahead of print] PubMed PMID: 23605440.
 4. Stevens JB, Horne SD, Abdallah BY, Ye CJ, Heng HH. Chromosomal instability and transcriptome dynamics in cancer. *Cancer Metastasis Rev*. 2013 Apr 18. [Epub ahead of print] PubMed PMID: 23595307.
 5. Heng HH, Liu G, Stevens JB, Abdallah BY, Horne SD, Ye KJ, Bremer SW, Chowdhury SK, Ye CJ. Karyotype heterogeneity and unclassified chromosomal abnormalities. *Cytogenet Genome Res*. 2013;139(3):144-57. doi: 10.1159/000348682. Epub 2013 Apr 3. Review. PubMed PMID: 23571381.
 6. Stevens JB, Abdallah BY, Liu G, Horne SD, Bremer SW, Ye KJ, Huang JY, Kurkinen M, Ye CJ, Heng HH. Heterogeneity of cell death. *Cytogenet Genome Res*. 2013;139(3):164-73. doi: 10.1159/000348679. Epub 2013 Apr 3. Review. PubMed PMID: 23548436.
 7. Horne SD, Abdallah BY, Stevens JB, Liu G, Ye KJ, Bremer SW, Heng HH. Genome constraint through sexual reproduction: application of 4D-Genomics in reproductive biology. *Syst Biol Reprod Med*. 2013 Jun;59(3):124-30. doi: 10.3109/19396368.2012.754969. Epub 2013 Jan 8. PubMed PMID: 23294443.
 8. Horne SD, Stevens JB, Abdallah BY, Liu G, Bremer SW, Ye CJ, Heng HH. Why imatinib remains an exception of cancer research. *J Cell Physiol*. 2013 Apr;228(4):665-70. doi: 10.1002/jcp.24233. Review. PubMed PMID: 23018746.

Reportable outcomes

1. Preliminary samples that include 15 GWI patients and 35 controls indicate significant correlation with genomic instability identified in GWI patients when compared to controls.
2. Potential biomarkers have been identified that might be present in GWI patients that could be used for diagnosis and possible disease severity/progression prognosis correlation.
3. The new correlation between genome instability and gene expression dynamics has been established.
4. There is some overlap between GWI and CFS in terms of profiling by genome instability.

Conclusions:

- a. The patient samples analyzed so far display elevated chromosomal aberrations as determined by SKY analyses. This is a very important finding. With the examination of additional patients followed by statistical analyses, we anticipate that we will be able to report this exciting new link between genomic instability and GWI.
- b. Lab experimental protocols are successful and are being further refined for use in GWI patient populations. One example of this is the use of interphase FISH to confirm the involvement of aneuploidy, as the detection of polyploidy has been higher in patients.
- c. We have discovered data differences in control groups. Due to this finding we also will compare more samples between the military and non-military populations. The military cases with CFS might be the contributing factor.
- d. Due to the identification of potential biomarkers, we are developing additional cytogenetic characterization to systematically link potential sub-groups of specific types of chromosomal aberrations. Methodology applications are also being considered for use in commonly associated GWI comorbidities (e.g. Myalgic Encephalomyelitis/Chronic Fatigue Syndrome).
- e. New controls will be added to the study and we will complete work on additional GWI patient samples. The VA clinic in Detroit has supplied 15 GWI patients to date and we plan to use our mailing list to contact additional veterans/patients to collect the needed additional GWI samples.

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1. Stevens JB, Liu G, Abdallah BY, Horne SD, Ye KJ, Bremer SW, Ye CJ, Krawetz SA, Heng HH. Unstable genomes elevate transcriptome dynamics. *Int J Cancer*. 2013 Oct 10. doi: 10.1002/ijc.28531. [Epub ahead of print] PubMed PMID: 24122714.
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